# Lactation Performance, Milk Fatty Acid Composition, and Blood Lipid Profile of Lactating Buffaloes in Response to Dietary Soybean and Linseed Oils

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## ABSTRACT

There is an awareness in augmenting conjugated linoleic acid (CLA) and n-3 fatty acids (FA), while lowering saturated FA (SFA) in ruminant milk due to their advantageous health benefits for humans. Therefore, we aimed to investigate the effects of soybean and linseed oils on milk yield, milk composition, milk FA profile, and blood lipid metabolites. Eighteen multiparous Egyptian buffaloes fed corn silage and clover hay-based diet were allocated at random for body weight, days in milk, and milk yield into 3 groups of 6 buffaloes each using a randomized block design. Dietary treatments consisted of three concentrate supplements containing no additional plant oil (CON) or containing soybean oil (SO) or linseed oil at a level of 2.6% of dry matter intake (DMI) (400 g/head/d; 25.6 g/kg DMI). Moderate dietary inclusion of SO or LO had no detrimental effects on DMI, milk production, or milk fat per cent. Milk protein, lactose, ash, and total solids concentrations were increased by LO supplementation. The LO diet decreased milk total SFA content (C10:0, C12:0, C14:0, C16:0), augment monounsaturated FA (MUFA), 18:3n-3 FA, CLA, and polyunsaturated FA, as well as lower atherogenicity index and n-6:n-3 compared to CON. On the other hand, the SO diet increased milk fat content of n-6 FA, CLA, MUFA, and n-6:n-3, and decreased the atherogenicity index compared to CON. LO supplementation had no effect on serum concentrations of triglyceride, total lipids, HDL, and LDL. However, the SO group tended to have greater serum total lipids and LDL concentrations and significantly elevated level of serum cholesterol. In conclusion, dietary inclusion of LO in the concentrate supplement of dairy buffaloes could enrich the nutritive value of milk with advantageous FA (n-3 FA, MUFA, and CLA) and lower the atherogenicity index and n6 to n3 ratio without triggering any adverse effects on buffalo performance.

*Keywords: buffaloes; plant oil; milk composition; milk fatty acid profile; blood metabolites*

## INTRODUCTION

Buffaloes are commonly allocated all over Asia, the Middle East, Europe, China, and South America. Buffaloes are considered as the second milk producing animal world-wide. The average milk production of lactating buffaloes in 2009 was approximately 90 million tons representing about 13% of the total world milk production with an annual rise of 3.1% in comparison to 1.3% annual rise in dairy cow milk production (IDF, 2010). Buffalo milk plays a vital role in the human diet predominantly in the developing countries and is favored by the Egyptian consumers because of its white color, high-fat content, and good flavor (El-Salam & El-Shibiny, 2011). Compared with cow milk, buffalo milk is rich in nearly all the chief milk nutrients such as fat, protein, lactose, casein, and ash contents (El-Salam & El-Shibiny, 2011). Buffalo's milk fat contained higher total saturated fatty acids (SFA, C12:0, C14:0, and C16:0) and lower monounsaturated fatty acids (MUFA) con-

centrations than the cow's milk fat (Ménard *et al.*, 2010; Gagliostro *et al.*, 2015).

Milk and dairy products are vital constituents of healthy human diets because of their ability to supply their diets with essential macro- and micronutrients (Lordan *et al.*, 2018). The consumption of dairy products is accompanied by several adverse health problems due to their SFA content, which may lead to the elevated blood low-density lipoprotein (LDL), and consequently may increase the risk of cardiovascular disease and lower insulin sensitivity in humans (Lordan *et al.*, 2018). However, C18:0 fatty acid (FA) is considered as a neutral FA compared to MUFA because it increases circulating LDL-cholesterol concentrations (Shingfield *et al.*, 2008). Besides, several branched-chain SFA and other bioactive lipids in the milk fat are thought to exhibit anticarcinogenic properties and positive effects concerning the decrease prevalence of chronic diseases in humans (Shingfield *et al.*, 2008; Halmemies-Beauchet-Filleau *et al.*, 2011).

Due to the potential health benefits for humans, there is an awareness in augmenting cis-9, trans-11 conjugated linoleic acid (CLA) and polyunsaturated n-3 FA, while decreasing medium-chain FA in the milk of dairy cows through plant oils supplementation (Shingfield *et al*., 2013; Kairenius *et al*., 2018; Mahdavi *et al*., 2019). To our knowledge, the effect of plant oils varying in linoleic or linolenic acid concentrations (soybean oil and linseed oil) on milk composition, milk fatty acids profile, and blood lipid constituents in lactating buffaloes are very limited. Therefore, the objectives of the present study were to enrich the milk of lactating buffaloes with beneficial FA (C18:3 n-3, CLA) and decrease milk n6:n3 ratio through supplementation of a moderate amount of soybean oil (SO) or linseed oil (LO) in their diets without inducing negative effects on total dry matter intake (DMI). Furthermore, a direct comparison of the effects of SO vs. LO was made to determine the suitable plant oil in the diet of lactating buffaloes. The hypothesis tested was that dietary inclusion of plant oils could modify milk FA profile depending on whether linolenic acid (C18:3 n-3) or linoleic acid (C18:2 n-6) is the main polyunsaturated FA (PUFA), and that oil rich in C18:3 n-3 could increase n-3 PUFA and CLA in the milk of buffaloes.

## MATERIALS AND METHODS

### Animals, Experimental Design, and Diets

The experiment was performed at Buffalo Research Unit, Animal Production Department, Faculty of Agriculture, Menoufia University (Menoufia, Egypt). The location is at the latitude of 30.5503° N and 31.0106° E. All the experimental procedures were approved by the Animal Ethics Committee (Menoufia University) and consistent with Egyptian rules for animal welfare. Eighteen multiparous Egyptian buffaloes (second to fourth parity) averaging 600 ± 33 kg of body weight (BW), 96 ± 13 days in milk and producing 8.09 ± 0.72 kg

of milk/d, were blocked according to days in milk, milk yield, and BW into 3 groups of 6 buffaloes each. The experimental design was a randomized block design with 6 replicates per treatment. Dietary treatments consisted of three concentrate supplements containing no additional lipid (CON), soybean oil (SO), or linseed oil (LO). Dairy buffaloes were offered corn silage, clover hay (long-stem), and a cereal-based concentrate (forage to concentrate ratio of 70:30, on a DM basis). All buffaloes were fed a basal ration with 5 kg/d a cereal-based concentrate (with or without oil). The concentrate was offered as equal meals at 05:00 AM and 17:00 PM. The level of oil included in the concentrate calculated as a percent to DMI was 2.6% (400 g/head/d, 25.6 g/kg DMI). The ingredients, chemical, and fatty acid compositions of the feeds and concentrates used in the experiment are shown in Tables 1, 2, and 3. The supplemented oil, as a constituent of concentrate, was mixed well with wheat bran and then was mixed with the whole concentrate ingredients immediately before feeding. All lactating buffaloes were individually fed in accordance with the experimental treatments. The study was conducted for 8 weeks, involving the first 14 days as an adaptation period, and the next 6 weeks as a measurable period. Animals were housed in individual tie stalls with free access to water and were milked twice per day at 06:00 AM and 18:00 PM using portable milking machine.

### Measurements, Sample Collection, and Chemical Analysis

At the end of the study, feed samples were taken for chemical analysis. Samples were subjected to proximate chemical analysis according to the procedures of AOAC (2005) for dry matter (DM), crude protein (CP), ether extract (EE), and ash. Neutral detergent fiber (NDF) was measured using the method described by Van Soest *et al*. (1991). The chemical analysis was expressed on DM basis. Dairy buffaloes were milked twice

Table 1. Formulation and ingredient composition of experimental concentrates

| Ingredient, g/kg DM | Concentrate[a] | | |
|---|---|---|---|
| | CON | SO | LO |
| Yellow corn, ground | 500 | 340 | 340 |
| Un-decorticated CSM | 170 | 170 | 170 |
| Soybean meal | 80 | 80 | 80 |
| Wheat bran | 220 | 300 | 300 |
| Soybean oil | - | 80 | - |
| Linseed oil | - | - | 80 |
| Limestone | 10 | 10 | 10 |
| Sodium bicarbonate | 10 | 10 | 10 |
| Sodium chloride | 7 | 7 | 7 |
| Mineral and vitamin mixture[b] | 3 | 3 | 3 |

Note: [a]Refers to experimental concentrates containing no additional oil (CON), soybean oil (SO), or linseed oil (LO).
[b]Mineral and vitamin mixture: provided per kg of concentrate including 5000 IU of vitamin A, 2200 IU of vitamin D3, 15 IU of vitamin E, 9.5 g of K, 2.4 g of Mg; 2.1 g of Na, 3.4 g of Cl, 3.2 g of S, 0.16 mg of Co, 100 mg of Cu, 1.3 mg of I, 64 mg of Mn, 64 mg of Zn, 64 mg of Fe, and 0.45 mg of Se.

Table 2. The chemical and fatty acid compositions of concentrates used in the experiment

| Item | Concentrate[a] | | |
|---|---|---|---|
| | CON | SO | LO |
| Chemical composition, g/kg DM | | | |
| Dry matter | 900 | 912 | 913 |
| Crude protein | 184 | 183.5 | 184.2 |
| Metabolizable energy, MJ/kg | 12.36 | 13.57 | 13.71 |
| Neutral detergent fiber | 201.0 | 217.8 | 219.7 |
| Ether extract | 34.1 | 105.5 | 101.3 |
| Ash | 42.9 | 45.3 | 44.6 |
| Fatty acid composition, g/100g fatty acids | | | |
| C16:0 | 13.93 | 15.49 | 14.95 |
| C18:0 | 1.56 | 2.07 | 2.13 |
| C18:1 | 23.50 | 24.37 | 24.26 |
| C18:2 n6 | 37.67 | 33.58 | 31.50 |
| C18:3 n3 | 7.43 | 9.17 | 13.50 |
| C20:0 | 0.41 | 0.43 | 0.38 |

Note: [a]Refers to experimental concentrates containing no additional oil (CON), soybean oil (SO), or linseed oil (LO).

Table 3. The chemical and fatty acid compositions of forages and oils used in the experiment

| Item | Forage | | Oil | |
|---|---|---|---|---|
| | Corn silage | Clover hay | Soybean | Linseed |
| Chemical composition, g/kg DM | | | | |
| Dry matter | 353 | 880 | - | - |
| Crude protein | 81.0 | 157.0 | - | - |
| Metabolizable energy, MJ/kg | 11.30 | 9.60 | 35.15 | 36.82 |
| Neutral detergent fiber | 450 | 520 | - | - |
| Ether extract | 29.5 | 19.5 | 1000 | 1000 |
| Ash | 51 | 80 | - | - |
| Fatty acid composition, g/100 g fatty acids | | | | |
| C16:0 | 22.07 | 16.68 | 12.44 | 5.71 |
| C18:0 | 9.54 | 10.77 | 4.95 | 5.72 |
| C18:1 | 9.75 | 21.24 | 24.75 | 22.18 |
| C18:2 n6 | 17.79 | 15.24 | 49.6 | 14.67 |
| C18:3 n3 | 15.60 | 43.09 | 7.90 | 49.55 |
| C20:0 | 1.1 | 0.39 | 0.33 | 0.16 |

daily, and milk yields were recorded individually along 6 weeks. The collected milk samples for 2 consecutive days per week were analyzed for protein, lactose, fat, ash, and total solids concentrations using an ultrasonic milk analyzer (Lactoscan, Model MCC, Milk Analyzer, Bulgaria). Average daily yields (g/d) of milk components were calculated for each buffalo by multiplying milk production by the content of milk component (g/kg). The milk gross energy was calculated using the formula of Tyrell & Reid (1965). Energy-corrected milk (ECM) was calculated using the equation of Sjaunja *et al*. (1991). The milk energy output (MJ/d) was calculated by multiplying milk energy (MJ/kg) by milk production (kg/d).

Milk samples were collected from individual buffaloes at the end of the experiment and stored at −20°C until analyzed for FA composition. The FA composition was determined by conversion of oil to FA methyl esters by adding n-hexane (950 μL), oil (50 mg) and sodium methoxide (50 μL) according to Cocks & Van Rede (1966) using a gas chromatograph (Model GC-14A, Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionization detector and a polar capillary column (BPX70, 0.25; SGE Incorporated, USA). The FA methyl esters peaks were recognized by comparing their retention times with the authentic FA methyl ester standards. The FA relative percent was calculated depending on the peak area of FA to the total peak area of all FA in the sample.

Blood samples from the jugular vein of each buffalo were collected at the end of the experiment into collection tubes (Venoject, Terumo Europe Ltd., Leuven, Belgium). Separations of serum from coagulant blood were conducted immediately within an hour by centrifugation for 15 minutes at 3000 rpm (Multifuge IS-R, D-37520 Osterode, Germany). Collected serums were stored at -20°C for further analysis. Total triglycerides, total lipids, high-density lipoprotein (HDL), LDL, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured as explained by Selim & Hussein (2020).

### Statistical Analysis

Experimental data were analyzed by ANOVA using the Mixed procedure of SAS (version 9.2, SAS Institute, Cary, NC) with a model that included the buffalo as a random effect and week and treatment as fixed effects. Sums of squares for dietary treatments were then separated into a single degree of freedom contrasts to test their significances as follows: (1) plant oils in the diet (control vs. SO + LO), and (2) comparison of plant oil PUFA sources (SO vs. LO). Arithmetic means are reported, and treatment effects declared significant at $p<0.05$. Data are expressed as mean ± SEM.

### RESULTS

#### Dry Matter and Nutrient Intakes

Dry matter, ME, CP, EE, NDF, and ash concentrations of corn silage and clover hay were normally in the ranges reported (Table 3). Corn silage was abundant in C16:0, while clover hay was rich in C18:3 n3 (Table 3). Soybean oil was rich in C18:2 n6, whereas LO was high in C18:3 n3 (Table 3). Buffaloes consumed all the concentrates fed. Total DMI, forage intake, and NDF intake was not altered by the dietary inclusion of SO or LO in concentrate supplements comprising approximately 2.6% of the diet DM (400 g/head/d or 25.6 g/kg DM) (Table 4).

#### Milk Yield and Composition

Daily yields of milk, fat, protein, and lactose were not altered by experimental treatments (Table 4). The dietary SO or LO inclusion at 400 g/d or 25.6 g/kg DMI had no effect on milk yield (Table 4). Milk protein ($p<0.01$), lactose ($p<0.01$), ash ($p<0.05$), and total solids ($p<0.01$) concentrations were strongly affected by LO supplementation (Table 4), characterized by greater values compared to those of the control group. There was no significant difference in milk composition between

Table 4. Nutrient intakes, milk production, and milk composition of dairy buffaloes treated by soybean oil or linseed oil

| Variables[c] | Treatments[a] | | | SEM | Significance[b] | |
|---|---|---|---|---|---|---|
| | CON | SO | LO | | CON vs. others | SO vs. LO |
| Intake | | | | | | |
| Dry matter, kg/d | 15.80 | 15.65 | 15.70 | 0.39 | 0.84 | 0.86 |
| Corn silage, kg DM/d | 6.20 | 6.15 | 6.20 | 0.34 | 0.75 | 0.76 |
| Clover hay, kg DM/d | 4.60 | 4.50 | 4.50 | 0.20 | 0.94 | 0.99 |
| Metabolizable energy, MJ/d | 176.11 | 180.55 | 181.81 | 4.53 | 0.44 | 0.65 |
| Neutral detergent fiber, kg/d | 6.19 | 6.20 | 6.23 | 0.18 | 0.54 | 0.69 |
| Yield | | | | | | |
| Milk yield, kg/d | 8.37 | 8.25 | 8.04 | 0.28 | 0.53 | 0.75 |
| ECM, kg/d | 13.76 | 12.53 | 13.93 | 0.817 | 0.26 | 0.29 |
| Milk fat, g/d | 782.35 | 678.19 | 788.28 | 51.52 | 0.13 | 0.16 |
| Milk protein, g/d | 303.05 | 302.40 | 307.82 | 13.45 | 0.86 | 0.97 |
| Milk lactose, g/d | 349.10 | 354.96 | 357.91 | 16.30 | 0.86 | 0.98 |
| Concentration in milk | | | | | | |
| Total solids, % | 17.88[ab] | 16.97[b] | 18.87[a] | 0.406 | 0.009 | 0.003 |
| Fat, % | 9.34[ab] | 8.20[b] | 9.80[a] | 0.33 | 0.008 | 0.007 |
| Protein, % | 3.62[b] | 3.67[b] | 3.82[a] | 0.027 | 0.003 | 0.01 |
| Lactose, % | 4.17[b] | 4.30[ab] | 4.45[a] | 0.06 | 0.004 | 0.05 |
| Ash, % | 0.75[b] | 0.77[ab] | 0.80[a] | 0.01 | 0.04 | 0.05 |
| Milk energy content, MJ/kg | 5.11[ab] | 4.72[b] | 5.39[a] | 0.145 | 0.01 | 0.009 |
| Milk energy output, MJ/d | 42.83 | 38.97 | 43.36 | 2.562 | 0.26 | 0.28 |
| Milk efficiency | | | | | | |
| Milk/DMI | 0.547 | 0.545 | 0.529 | 0.019 | 0.60 | 0.43 |
| ECM/DMI | 0.90 | 0.82 | 0.91 | 0.053 | 0.25 | 0.14 |

Note: [a]Refers to experimental concentrates containing no additional oil (CON), soybean oil (SO), or linseed oil (LO).
[b]Significance of effects due to plant oil in the diet (CON vs. SO and LO); source of PUFA in the diet (SO vs. LO); Means in the same row with different superscripts differ significantly (p<0.05); SEM= standard error of the mean.
[c]DMI= dry matter intake; ECM= energy corrected milk. Milk energy content (MJ/kg)= 4.184 × [(41.63 × fat (g/kg) + 24.13 × protein (g/kg) + 21.60 × lactose (g/kg) – 117.2)/10000] × 2.204 according to Tyrell & Reid (1965). ECM (kg/d)= milk (kg/d) × [38.3 × fat (g/kg) + 24.2 × protein (g/kg) + 16.54 × lactose (g/kg) + 20.7]/3140 according to Sjaunja et al. (1991).

SO and control. The LO supplementation significantly increased (p<0.01) milk lactose percentage (4.45% vs. 4.17 %) compared to control (Table 4).

## Milk Fatty Acid Profile

Data of milk fatty acids composition is presented in Table 5. Compared with the control group, buffaloes fed diets supplemented with LO or SO led to the increase in the milk fat concentrations of C4:0 and C6:0, and decrease C10:0, C12:0, C14:0, and C16:0 (p<0.001). An increased milk fat C18:0 (p<0.001) was also observed as the buffaloes were fed diets containing LO or SO diets compared to those fed the control diet (Table 5). The animals supplemented with LO or SO at a level of 400 g/d resulted in greater C18:2, C18:1, and C20:0 (p<0.001) than those fed the control diet. Milk fat concentration of C18:3 was reported to be higher (p<0.001) in the LO group than the other groups. There was a tendency (p= 0.05) of higher C20:1 concentration in the milk fat of buffaloes fed a diet supplemented with LO than those fed the control diet.

Milk samples obtained from SO- or LO-supplemented buffaloes with high n6- FA and CLA concentrations were also characterized by low atherogenicity index (lower index by about 24.19% and 47.65% for

SO and LO, respectively compared to control (p<0.001). Buffaloes fed LO had reduced milk SFA (59.73% vs. 66.70 and 68.59% for SO and control, respectively) and improved milk PUFA (6.03%) compared to the SO and control groups (2.53 and 2.93%, respectively), which was associated with the increasing PUFA to SFA ratio (p<0.001). The percentage of total MUFA obviously increased (p<0.001) as the level of LO or SO included up to 400 g/d that resulted in an increase (p<0.001) in the milk fat MUFA:SFA of these buffaloes. The greater total proportion of n-3 FA in the milk fat of lactating buffaloes fed diets supplemented with LO led to a decrease in the ratio of n-6 to n-3 FA milk fat (p<0.001). However, a higher proportion of total n-6 FA in the milk fat of the animals fed SO led to an increase of n-6:n3 FA in the milk fat of these buffaloes (p<0.001).

## Blood Lipid Profile and Hepatoprotective Activity

Buffaloes fed diets containing 400 g/d of SO showed a greater total cholesterol level (p<0.05) in the serum than those fed the CON diets (Table 6). A trend for higher serum total lipids (p=0.05) and LDL (p=0.08) was recorded in the buffaloes fed SO than those fed the CON diet (Table 6). There were no significant differences in serum total lipids, cholesterol, HDL, and LDL

Table 5. Milk fatty acids composition (g/100 g fatty acids) of dairy buffaloes treated by soybean oil or linseed oil

| Fatty acids[c] | Treatments[a] | | | SEM | Significance[b] | |
|---|---|---|---|---|---|---|
| | CON | SO | LO | | CON vs. others | SO vs. LO |
| C4:0 | 0.07[c] | 0.48[a] | 0.29[b] | 0.005 | <0.001 | <0.001 |
| C6:0 | 0.30[c] | 0.72[a] | 0.59[b] | 0.008 | <0.001 | <0.001 |
| C8:0 | 0.38 | 0.44 | 0.37 | 0.049 | 0.37 | 0.38 |
| C10:0 | 1.11[a] | 0.94[b] | 0.75[c] | 0.032 | <0.001 | 0.003 |
| C12:0 | 1.80[a] | 1.50[b] | 1.20[c] | 0.024 | <0.001 | <0.001 |
| C14:0 | 10.12[a] | 8.06[b] | 6.56[c] | 0.244 | <0.001 | 0.002 |
| ∑ C14:1 | 0.613 | 0.520 | 0.407 | 0.332 | 0.82 | 0.94 |
| C16:0 | 35.87[a] | 30.43[b] | 23.57[c] | 1.045 | <0.001 | 0.001 |
| ∑ C16:1 | 0.04[a] | 0.01[b] | 0.05[a] | 0.003 | <0.001 | <0.001 |
| C17:0 | 0.97[a] | 0.87[a] | 0.71[b] | 0.034 | 0.001 | 0.008 |
| C18:0 | 15.29[c] | 19.94[b] | 21.61[a] | 0.212 | <0.001 | 0.001 |
| ∑ cis C18:1 | 3.56 | 3.28 | 3.93 | 0.219 | 0.57 | 0.33 |
| ∑ trans C18:1 | 23.81[c] | 26.48[b] | 29.29[a] | 0.476 | <0.001 | 0.001 |
| ∑ C18:1 | 27.37[c] | 29.76[b] | 33.22[a] | 0.588 | <0.001 | 0.003 |
| C18:2 | 1.75[c] | 2.07[b] | 2.78[a] | 0.098 | <0.001 | 0.001 |
| CLA (conjugated linoleic acid) | 0.35[c] | 0.49[b] | 0.55[a] | 0.021 | 0.001 | 0.002 |
| C18:3 | 1.18[b] | 0.46[c] | 3.25[a] | 0.041 | <0.001 | <0.001 |
| C20:0 | 2.68[c] | 3.32[b] | 4.08[a] | 0.065 | <0.001 | <0.001 |
| ∑ C20:1 | 0.17 | 0.29 | 0.32 | 0.060 | 0.05 | 0.82 |
| Summary | | | | | | |
| ∑ SFA | 68.59[a] | 66.70[a] | 59.73[b] | 1.721 | 0.005 | 0.007 |
| ∑ MUFA | 28.19[c] | 30.57[b] | 33.99[a] | 0.409 | <0.001 | <0.001 |
| ∑ PUFA | 2.93[b] | 2.53[b] | 6.03[a] | 0.139 | <0.001 | <0.001 |
| MUFA/SFA | 0.41[c] | 0.46[b] | 0.59[a] | 0.007 | <0.001 | <0.001 |
| PUFA/SFA | 0.043[b] | 0.038[b] | 0.100[a] | 0.001 | <0.001 | <0.001 |
| n6/n3 | 1.48[b] | 4.52[a] | 0.855[c] | 0.111 | <0.001 | <0.001 |
| Estimated Δ9-desaturase activity (Fatty acid ratios) | | | | | | |
| C14:1:C14:0 | 0.061 | 0.066 | 0.063 | 0.040 | 0.99 | 0.98 |
| C16:1:C16:0 | 0.001[b] | 0.0003[b] | 0.002[a] | 0.0002 | <0.001 | <0.001 |
| Atherogenicity index | 2.77[a] | 2.10[b] | 1.45[c] | 0.045 | <0.001 | <0.001 |

Note: [a]Refers to experimental concentrates containing no additional oil (CON), soybean oil (SO), or linseed oil (LO).
[b]Significance of effects due to plant oil in the diet (CON vs. SO and LO); source of PUFA in the diet (SO vs. LO); Means in the same row with different superscripts differ significantly (p<0.05); SEM= standard error of the mean.
[c]CLA, conjugated linoleic acid (trans-10, cis-12 C18:2 and cis-9, trans-11 C18:2); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 6. Blood biochemical constituents of dairy buffaloes treated by soybean oil or linseed oil

| Variables[c] | Treatments[a] | | | SEM | Significance[b] | |
|---|---|---|---|---|---|---|
| | CON | SO | LO | | CON vs. others | SO vs. LO |
| Total triglycerides, mg/dL | 23.76 | 26.48 | 24.50 | 2.275 | 0.50 | 0.67 |
| Total lipids, mg/dL | 319.23[b] | 412.82[a] | 355.13[ab] | 29.151 | 0.05 | 0.20 |
| Cholesterol, mg/dL | 175.45[b] | 257.05[a] | 212.50[b] | 12.856 | 0.002 | 0.03 |
| HDL, mg/dL | 104.23 | 114.84 | 105.77 | 9.417 | 0.51 | 0.62 |
| LDL, mg/dL | 103.28 | 146.21 | 134.39 | 15.767 | 0.08 | 0.74 |
| AST, U/L | 57.00 | 59.50 | 55.60 | 1.633 | 0.53 | 0.12 |
| ALT, U/L | 36.90 | 38.60 | 34.60 | 1.352 | 0.65 | 0.33 |

Note: [a]Refers to experimental concentrates containing no additional oil (CON), soybean oil (SO), or linseed oil (LO).
[b]Significance of effects due to plant oil in the diet (CON vs. SO and LO); source of PUFA in the diet (SO vs. LO); Means in the same row with different superscripts differ significantly (p<0.05); SEM= standard error of the mean.
[c]HDL= high-density lipoprotein; LDL= low-density lipoprotein; AST= aspartate aminotransferase; ALT= alanine aminotransferase.

between the LO-supplemented group and the control group (Table 6). Serum liver enzymes, including AST and ALT, were not altered by the treatments (Table 6).

## DISCUSSION

### Dry Matter and Nutrient Intakes

In the current study, total DMI, forage intake, and NDF intake were not different by the relatively moderate inclusion of SO or LO in concentrate supplements. These findings are in accordance with the most literature reports (Dirandeh *et al.*, 2013; Mach *et al.*, 2013; Suksombat *et al.*, 2016) that showed slight effects of type and concentration of lipid supplement on DMI when its inclusion level was below 6% of DM. Meanwhile, at greater inclusion levels, plant oils may persuade adverse effects on DMI (Huhtanen *et al.*, 2008), but not in all situations (Shingfield *et al.*, 2008). Martin *et al.* (2008) and Lerch *et al.* (2012) reported that dairy cows fed with a diet supplemented with LO (5.7% or 3% DMI, respectively) in the basal diet had lower DMI. A recent study by Suksombat *et al.* (2016) concluded that supplementation of lactating dairy cows with LO at 2.9% DM in the basal diet (500 g/d) maintaining a 56:44 roughage to concentrate ratio did not alter feed and nutrient intakes. The meta-analysis by Mahdavi *et al.* (2019) showed that DMI was reduced as a result of SO supplementation in the diets of dairy cows. In the present study, buffaloes were fed LO or SO at about 25.6 g/kg of DMI (400 g/head/d), may not be high enough to reduce DM and nutrient intakes. Furthermore, the lack of variations in DMI suggested that feed palatability was not altered by LO or SO inclusion. Unfortunately, there is no any previous researches on the effects of the dietary inclusion of LO or SO on DMI of lactating buffaloes to compare with the findings reported herein. The reduction in DMI of dairy cows fed plant oils, such as LO, SO, fish oil or sunflower oil, have been attributed to the detrimental effects of unsaturated FA on rumen microorganisms causing a decrease of ruminal fiber digestibility and increase of cholecystokinin and glucagon-like peptide 1 activities (Jenkins & Harvatine, 2014; Kairenius *et al.*, 2018; Mahdavi *et al.*, 2019).

### Milk Yield and Composition

The impacts of oil supplements on milk yield are different and known to be affected by the inclusion level of fat, degree of unsaturation, and diet composition (Halmemies-Beauchet-Filleau *et al.*, 2011; Suksombat *et al.*, 2014 & 2016). Successful SO or LO inclusion at 400 g/d or 25.6 g/kg DMI had no effect on milk production in the present study. Likewise, unaffected milk production was reported in dairy cows fed LO-supplemented diet at 500 g/d (Suksombat *et al.*, 2014 & 2016). However, very recent studies on lactating goats (Kholif *et al.*, 2016 & 2018) and dairy cows (Mahdavi *et al.*, 2019) revealed an increase in milk yield as a result of SO supplementation. They attributed this increase in milk production to the enhanced nutrient digestion and ruminal fermentation, as well as the increased energy density and energy

intake. In contrast, supplementation of 800 g/d fish oil + LO at a ratio of 1:3 (w/w) (Brown *et al.*, 2008) or 5.7% LO or extruded linseed to corn silage-based diet of dairy cows (Martin *et al.*, 2008) had reduced milk production. The depression of DMI and/or nutrient digestibility (especially fiber) as a result of disturbances in rumen function, rumen fermentation, and a shift of the digestion site from the rumen to the intestine triggered by a high dietary intake of lipids (i.e.,>5% of DMI) were the main reasons for the reduced milk yield (Brown *et al.*, 2008; Martin *et al.*, 2008). In the current study, moderate amounts of LO or SO in the diet (400 g/head/d; 25.6 g/kg DM; 2.6% DMI) did not affect DM or nutrient intakes, which may contribute to the unchanged milk yield.

In the present study, milk fat concentration was not altered by dietary LO or SO supplementation. On the other hand, many recent studies (Kholif *et al.*, 2016 & 2018; Mahdavi *et al.*, 2019) recorded a reduction in the milk fat percent as a result of SO supplementation due to a decrease of fiber digestion and thus ruminal acetate production. Angulo *et al.* (2012) reported that dairy cows fed with a diet supplemented with 3.1% LO + docosahexaenoic acid Gold algae or sunflower oil + docosahexaenoic acid Gold algae, compared to those fed a control diet with 3.1% protected saturated fat, had decreased milk fat. A reduction of milk fat is more probably occurred with the inclusion of non-protected fat in corn silage-based diet, but this reduction was minimized with the other forages, particularly alfalfa hay (Halmemies-Beauchet-Filleau *et al.*, 2011). Furthermore, the decreased milk fat percent in some other previously reported studies was triggered by the reduced DMI and fiber digestion, because of the elevated level of ingested LO (Martin *et al.*, 2008) and the decreased lipogenesis in the mammary gland (Chilliard *et al.*, 2007).

Regarding milk lactose, LO supplementation increased milk lactose percentage. This would be a result of the augmented propionate production since it is the precursor for gluconeogenesis and lactose synthesis (Boerman & Lock 2014; Kholif *et al.*, 2018; Vargas *et al.*, 2020). Milk lactose percent was not affected by SO supplementation, which agreed with the results reported by Mahdavi *et al.* (2019). Milk protein percentage was increased in LO, while there was no difference in daily yield of protein g/d between the treatment groups. Similarly, Gagliostro *et al.* (2015) observed the same findings when a blend of SO and LO was included in the diets of dairy buffaloes. On the other hand, the protein percentage was not affected by LO supplementation (Suksombat *et al.*, 2014 & 2016). The effect of plant oils on milk protein percentage has been variable. Inconsistency between studies in milk protein content in response to LO supplementation may be elucidated by the inclusion level of LO in the diet and the composition of the basal diet (Suksombat *et al.*, 2014 & 2016; Gagliostro *et al.*, 2015).

### Milk Fatty Acid Profile

The mammary gland is responsible for *de novo* synthesis for all C4:0 to C12:0, most of the 14:0, and half of 16:0 secreted in milk, whereas long-chain FA is

originated from the diet or adipose tissue (Chilliard *et al*., 2007). Lactating buffaloes fed SO or LO diets had lower milk fat concentrations of C10:0, C12:0, C14:0, and C16:0, while greater milk fat content of C4:0 and C6:0. These findings revealed the less *de novo* FA synthesis appeared with lactating buffaloes fed LO and SO diets. Consequently, feeding high PUFA plant oils are commonly associated with the decreased *de novo* short- and medium-chain FA synthesis (Brown *et al*. 2008; Halmemies-Beauchet-Filleau *et al*., 2011; Lerch *et al*. 2012; Suksombat *et al*., 2016; Kholif *et al*., 2016 & 2018). This reduction may be attributed to the suppressing effects of long-chain FA on the activity of mammary gland lipogenic enzymes such as acetyl-CoA carboxylase and the *de novo* synthesis of SFA in the mammary gland (Halmemies-Beauchet-Filleau *et al*., 2011; Suksombat *et al*., 2016). This is a vital issue from the point of view in animal nutrition. A reduction in milk contents of C14:0 and C16:0 from SO- and LO- buffaloes may be an optimistic target from a human health viewpoint since greater proportions of C12:0, C14:0, and C16:0 have been reported to be responsible for elevated plasma levels of cholesterol and LDL and the incidence of cardiovascular problems in humans consuming such FA (Simopoulos, 2016).

The observed increase in the milk fat C18:0 as lactating buffaloes was fed diets containing LO or SO in the current study, indicating that greater ruminal biohydrogenation of UFA in buffaloes fed LO followed by the SO diet than in buffaloes fed the control diet (McKain *et al*., 2010; Suksombat *et al*., 2016). The increase of milk contents of n-3 FA, predominantly C18:3, and CLA in the buffaloes fed the LO diet in the present study, was in line with the magnitude responses reported in the other studies (Lerch *et al*., 2012; Mach *et al*. 2013; Suksombat *et al*., 2016). On the contrary, Lock & Garnsworthy (2002) observed that plant oils rich in n-6 FA (e.g., SO) was more potential in the enrichment of CLA in the milk fat than oils rich in n-3 FA (e.g., LO). Enrichment of CLA concentration in milk was reported to be derived from incomplete biohydrogenation of linoleic acid by the rumen resulting an increase in the supply of C18:1 trans 11 for desaturation in the mammary gland or from endogenous synthesis from vaccenic acid via $\Delta^9$-desaturase activity in the mammary gland (Shingfield *et al*., 2008; Halmemies-Beauchet-Filleau *et al*., 2011). The endogenous synthesis of CLA in the mammary gland is considered as the main pathway in lactating cows (Shingfield *et al*., 2008; Halmemies-Beauchet-Filleau *et al*., 2011). Milk obtained from LO-supplemented buffaloes had greater n3- FA, CLA concentration, and lower atherogenicity index, suggesting that the consumption of its milk and dairy products has less harmful impacts regarding the atherosclerosis problems, being beneficially healthier for humans (Kholif *et al*., 2016; Suksombat *et al*., 2016).

Lactating buffaloes fed the LO diet had lower milk SFA and greater milk PUFA and n-3 FA, which was associated with the increasing ratio of PUFA to SFA and the decreasing ratio of n-6 to n-3 FA. However, a greater total n-6 FA in the milk fat of the buffaloes fed SO caused an increase in the ratio of n-6 to n3 FA milk

fat of these buffaloes. These findings were in agreement with recent studies (Halmemies-Beauchet-Filleau *et al*., 2011; Lerch *et al*., 2012; Kholif *et al*., 2016; Suksombat *et al*., 2016), which reported similar responses in milk FA profile when plant oils were used in dairy cows or goats. Mahdavi *et al*. (2019) reported that SO supplementation had no effect on C18:3 n-3 in milk fat because of its low concentration in SO. Although both of n-3 and n-6 FA are essential and have valuable impacts on human health, excessive levels of n-6 FA and very low levels of n-3 FA (increased n-6 to n-3 ratio) possibly interfering the way of the body to utilize n-3 FA and therefore limit their abundant health benefits (Simopoulos, 2016). LO supplementation could lower the ratio of n-6 to n-3 more than SO supplementation (Antonacci *et al*., 2018). The ratio of n6 to n-3 is desirable to be closer to 2:1 (Benbrook *et al*., 2013). Lowering n-6 to n3 FA ratio in milk and dairy products has been recommended to decrease the incidence of certain diseases in humans (Simopoulos, 2016).

## Blood Lipid Profile and Hepatoprotective Activity

All measured blood metabolites were within the normal values, as reported by Abd Ellah *et al*. (2013). Dietary supplementation of oils had no detrimental impacts on liver function, as serum ALT and AST levels were not altered by the dietary treatments. The current findings are in consistent with the results reported by Kholif *et al*. (2016). Feeding oils did not alter blood triglyceride concentration in lactating dairy cows (Ye *et al*., 2009) and dairy goats (Kholif *et al*., 2016). Feeding oil are known to increase the energy density of the diet and energy intake, which in turn changes lipid metabolism in animal tissues. The current findings showed a tendency towards higher serum total lipids and LDL concentrations without affecting triglycerides, suggesting that lipid supplementation triggered the elevated levels of blood triglyceride-rich lipoproteins (Kholif *et al*., 2016) since LDL is responsible for cholesterol transportation. Similarly, Ye *et al*. (2009) observed that feeding SO and LO to lactating dairy cows increased plasma LDL concentration.

## CONCLUSION

Supplementing lactating buffalo diets-based on corn silage and clover hay with LO up to 400 g/d could augment the nutritive value of milk with valuable FA (n-3 FA, MUFA, and CLA) and reduce atherogenicity index and n-6 to n-3 FA ratio without triggering any adverse effects on milk yield, milk composition or buffalo performance.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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